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Selection criteria for assessing drought tolerance in a segregating population of flax (*Linum usitatissimum* L.)

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Abstract

Using plant genotypes adaptable to water-deficit stress is an optimal strategy in sustainable agriculture. This study was conducted to assess the selection criteria for identifying high yielding-drought tolerant F_2 - derived F_3 flax families from a cross between Iranian genotype KO37 and the Canadian genotype SP1066. One hundred and nineteen $F_{2,3}$ were evaluated under drought stress and non-stress conditions using an 11*11 lattice design with three replications. Sixteen drought tolerance indices adjusted based on grain yield under drought stress and non-stress conditions were calculated. The presence of high variability for grain yield and irrigation water use efficiency in parental lines and $F_{2,3}$ families under both conditions indicated that the $F_{2,3}$ population or its advanced generations can be used in selection programs to increase drought tolerance and also to identify quantitative trait loci / genes related to grain yield and drought tolerance in flax. The results of biplot based on the PC_1 and PC_2 and triplot analysis based on STI index and grain yield in both stress and non-stress conditions introduced thirteen families as the most promising families for drought tolerance and therefore, their advanced generations can be used in future breeding programs to improve drought tolerance in flax.

Keyword: Biplot, Drought Tolerance, Flax, Selection Index, Water Use Efficiency

Introduction

Flax (*Linum usitatissimum* L., $2n = 2x = 30$) is a self-pollinating, diploid species and dual-purpose crop grown commercially for its seed oil as well as its stem fiber. Flax oil, rich in the omega-3 fatty acid (alpha linolenic acid), a precursor for many essential fatty acids of human diet, is being used in the fabrication of biodegradable products such as linoleum as well as for functional food and feed. The fiber from flax stem is highly valued for use in textiles such as linen and in insulations (Kulpa and Denart 1962; Marchenkov et al. 2003).

Drought is one of the most damaging abiotic stresses in arid and semi arid regions of the world that affects the seed yield and yield stability of oilseed crops. This is through alternations in metabolism and gene expression and cause declines in many traits such as root growth, leaf water potential, cell membrane stability, photosynthetic rate, photochemical efficiency as well as in quality and quantity of oil (Van Ginkel et al. 1998; Schroeder et al. 2001; Luan 2002; Biswas et al. 2011).

To overcome these limitations, strategies such as the use of suitable varieties adapted to stress conditions with high irrigation water use efficiency (IWUE) (Siahpoosh et al. 2011; Blum 2005) and using deficit irrigation management (Costa et al. 2007; Geerts and Raes 2009; Gheysari et al. 2009) have been suggested. However, in flax only a few studies have been accomplished on evaluating water deficit stress and IWUE and on identifying drought tolerant genotypes (Sharma et al. 2012; Yenpreddiwar et al. 2007).

Plant improvement for drought tolerance through identification of drought tolerant genotypes has been a goal in oilseed crops breeding programs. However, breeding for drought tolerance is genetically complicated because of its polygenic nature, difficulty of simultaneous selection of genes/QTLs involved (Yeo 1998; Flowers et al. 2000) and the lack of fast, reproducible screening techniques particularly under field conditions (Ramirez and Kelly 1998). The assessment of early generation segregating populations from crosses between parental lines contrasting for drought tolerance, under different water deficit stress conditions is one of the first steps to identify the chromosomal location of genes/QTLs related to drought tolerance.

Various quantitative criteria based on yield reduction under water deficit stress conditions in comparison to non-stress conditions have commonly been proposed to identify drought tolerant genotypes (Fischer and Maurer 1978; Clarke et al. 1992; Fernandez 1992; Byrne et al. 1995). Fernandez (1992) stated that SSI (stress susceptibility index) determines sensitive and tolerant families without attention to yield potential. It was reported that SSI is a useful indicator for wheat breeding where the stress is severe while MP (mean productivity), GMP, TOL (tolerance index), HM (harmonic mean) and STI (Stress tolerance index) are better if the stress is less severe (Akçura et al. 2011; Clarke et al. 1992 and Guttieri et al. 2001). The relative effectiveness of indices such as STI, MP and GMP in discriminating drought tolerant genotypes was reported in maize (Farshadfar and Sutka 2002, Jafaria et al. 2009; Hejazi et al. 2013), safflower (Pourdad 2008; Majidi et al. 2011), rapeseed (Yarnia et al. 2011) and wheat (Anwar et al. 2011; Nouraein et al. 2013; El-Rawy and Hassan 2014). Also, these indices were identified as suitable indices to select the high-yielding and drought tolerance families among F_3 and F_4 families of durum wheat (Golabadi et al. 2006) and F_5 families of rice (Rahimi et al. 2013). Fernandez (1992), in a three-dimensional representation of genotypes based on Y_s , Y_p and GMP, categorized

genotypes into four groups based on their performance in water deficit stress and non-stress environments; genotypes expressing uniform superiority in both water deficit stress and non-stress environments (Group A); genotypes with high performance in non-water deficit stress environments (Group B); genotypes with relatively high yield only in water deficit stress environments (Group C) and genotypes performing poorly in both water deficit stress and non-stress environments (Group D). The optimal selection criteria should distinguish group A from the other three groups (Fernandez 1992). According to this, STI and GMP indices were introduced as the best selection criteria for distinguishing tolerant and susceptible genotypes (Vegh 2013; Ramirez and Kelly 1998; Nouraein et al. 2013; Majidi et al. 2011). Nouraein et al. (2013) reported that GMP is more powerful in separating group A and has a lower susceptibility to different amounts of Y_s and Y_p ; So GMP will be bias when the difference between Y_s and Y_p is less.

Mohammadi et al. (2010) carried out a complete diallel cross using eight flax genotypes including four Iranian breeding lines (KO37, KH124, SE65 and AH92) and four Canadian genotypes (SP1066, CDC1774, Flanders and McGregor) and estimated genetic parameters for days to flowering, days to maturity, plant height, primary branches per plant, number of capsules per plant, number of seeds per capsule, seed yield per plant, 1000-seed weight, seed yield, oil yield and harvest index. The superiority of the parental line KO37 for oil yield, fatty acids, seed yield and other agronomic traits despite its short plant height was observed. As compared to KO37, SP10266 had lower mean values for most traits except for plant height. So, these genotypes were considered suitable for crossing and production of F_2 population. Also, eight flax genotype including KO37 and SP1066 were assayed under water deficit stress and non-stress field conditions in 2011 at the Research Farm of Isfahan University of Technology (RFIUT), Isfahan, Iran. Based on the result, KO37 was the most drought tolerant genotype with higher yield. On the other hand, SP1066 with a Canadian origin had taller height and a more erect stature.

Flax is a neglected oilseed crop of developing countries grown on marginal land with poor management. The poor yield of this crop is attributed to non availability of improved cultivars to suit the diverse agroclimatic conditions. Hence, development of high yielding and drought tolerant flax genotypes with high water use efficiency and productivity would greatly facilitate the cultivation of this crop in arid and semi-arid regions. This study was conducted to assess the selection criteria for identifying high yielding-drought tolerant F_2 - derived F_3 families from a cross between Iranian flax genotype KO37 and the Canadian genotype SP1066 under water deficit stress and non-stress field conditions.

Material and Methods

Plant materials

An F_2 population of 400 individuals from a cross between Iranian genotype KO37 and the Canadian cultivar SP1066 was generated (Mohammadi et al. 2010). The F_2 plants were grown in the field and the F_3 seeds were harvested in 2011. Among the F_2 plants only 119 produced sufficient seeds for a replicated field experiment. Therefore the seeds from these 119 F_2 plants were field evaluated in 2012.

Experimental site and design

The 119 F₃ families along with the parents were sown according to a lattice design (11*11) with three replications. Planting rows were 2m-long with 0.3 m distance between the rows with plant to plant distances of 2 cm. The experimental field was located in the Research Farm of Isfahan University of Technology (RFIUT), Isfahan, Iran (51° 32' E and 32° 22' N, 1630 m asl). The field soil was a Typic Haplargid with clay loam texture, pH 7.5 and 0.5% organic matter content. The mean annual precipitation was 110 mm and mean annual temperature was 15° C at RFIUT in 2012 (Table1). After sowing, fertilizers were applied at a rate of 10 kg N ha⁻¹ and 40 kg (NH₄)₃PO₄ ha⁻¹, and additional side dressing of 10 kg N ha⁻¹ was applied before flowering stage and weed was controlled manually. The mean of days to flowering and maturity was 64 and 93 days for KO37 (Iranian genotype).

Irrigation system

For irrigation a surface drip-tape system (Eurodrip S.A. Inc.¹) was used with the drippers 15 cm apart and 1.3 Lit h⁻¹ flow rate for each dripper, and one streamline for each crop row. For the first 55 days after sowing the irrigation time and amounts were the same for all the plots but after that the irrigation treatments were applied.

The irrigation schedule was determined using the FAO-PM equation and weather data (Allen et al. 1998). The reference crop evapotranspiration (ET₀) was calculated based on FAO-Penman-Monteith (FPM) equation (Allen et al. 1998), and the evapotranspiration of linseed (ET_C) was computed using ET₀ and recommended linseed crop coefficients (K_C) (Allen et al., 1998).

$$ET_0 = \frac{0.408\Delta(R_n - G) + \gamma \frac{900}{T + 273} u_2 (e_s - e_a)}{\Delta + \gamma(1 + 0.34u_2)} \quad (1)$$

$$ET_C = ET_0 \times K_C \quad (2)$$

Where ET₀ is the reference crop evapotranspiration (mm day⁻¹), R_n is the net radiation at the crop surface (MJ m⁻² day⁻¹), G is the soil heat flux density (MJ m⁻² day⁻¹), T is the mean daily air temperature at 2m height (°C), u₂ is the wind speed at 2 m height (m s⁻¹), e_s is saturation vapor pressure (kPa), e_a is actual vapor pressure (kPa), e_s-e_a is saturation vapor pressure deficit (kPa), Δ is slope vapor pressure curve (kPa °C⁻¹), and γ is psychometric constant (kPa °C⁻¹).

For no water deficit stress condition, irrigation was started when the management allowed depletion (P) of the available water reached $(1 \pm 0.05) \times 50\%$ for linseed (Allen et al. 1998). That is, when almost 50% of the total available water was depleted from root zone, the no water deficit stress treatment was irrigated. The threshold of the soil water content (θ_{IR}) in no water deficit stress condition was calculated as follows (Gheysari et al. 2009, Kiani et al. 2016):

$$\theta_{IR} = \theta_{FC} - (\theta_{FC} - \theta_{WP}) \times P \quad (3)$$

Where, θ_{FC} is soil water deficit content at field capacity (m³ m⁻³), and θ_{WP} is soil water content at wilting point (m³ m⁻³).

¹ - <http://www.eurodrip.gr/contactUs.aspx?affID=2&langID=2>

The maximum allowed depletion of water (MADW) (mm) from the root zone under no water stress treatment was calculated as follows (Gheysari et al. 2015):

$$\text{MADW} = (\theta_{\text{FC}} - \theta_{\text{IR}}) \times Z \quad (4)$$

Where, Z is the root zone depth (mm), in this study equal to 25 cm for first 30 days, and 40 cm for remind growing period. No water deficit stress plants were watered when the water content of the soil was 50% of the way to wilting (θ_{wp}) at either 25 cm or 40 cm soil depth, calculated based on total ET_c for flax and these soils. When $\sum ET_c$ (cumulative ET_c) after each irrigation event equaled MADW, irrigation was applied to the no water stress plants and irrigation depth was equal to MADW. For water stress treatment, irrigation interval was increased two times that of no water deficit stress treatment and irrigation depth was equal to MADW. During the growing season, for control of the irrigation treatments, the gravimetric method (Smith and Warrick 2007) was used to measure soil water content before irrigation.

Characters and Selection indices

Grain yield (gram per square meter) was determined for the parents and 119 F_3 families under water deficit stress and non-stress conditions and indicated as Y_s and Y_p , respectively. Also, irrigation water use efficiency (IWUE, gram per liter), calculated according to the formula: grain yield /applied water, was determined under water deficit stress and non-stress conditions and indicated as IWUEs and IWUEp, respectively. Drought tolerance indices were calculated using the relationships given in table2.

Statistical analysis

Because the relative efficiency of lattice design to randomized complete block design (RCBD) for grain yield (g m^{-2}) was nearly 100%, therefore the analysis of variance was conducted based on the RCBD for all calculated indices. The analysis of variance and principal component analysis for calculated indices was performed using SAS statistical program. The CORR SAS procedure was used to estimate correlations among indices. Cluster analysis of F_3 families was conducted using Wards method (Squared Euclidean) and the bi and triple plots were drawn using Stat Graphics software.

Result

Analysis of variance and mean comparison of indices

For non-water deficit stress condition the total of applied water including the irrigation and rain was 408.8 mm from sowing to harvest in 2012. The water deficit stress treatment received 25 percent less water than the control one. Time and amount of cumulative applied water including irrigation and rain and flax cumulative evapotranspiration represents the precise implementation of irrigation treatments (Fig. 1 and table1).

The analysis of variance indicated significant differences among the parents and F_3 families for Y_p , Y_s , IWUEp and IWUEs and all drought tolerance indices, except for $k1STI$ and $K2STI$ (Table 3). In non-water deficit stress condition, the mean of grain yield

per square meter for F₃ families, KO37 and SP1066 were 175, 250 and 130 g m⁻², respectively. These values were 98, 160 and 70 g m⁻² in water deficit stress condition. Among F₃ families, grain yield varied from 78 to 312 and from 32 to 168 g m⁻² in non-water deficit stress and water deficit stress conditions, respectively (Fig. 2). Mean grain yield of F₃ families, KO37 and SP1066 in water deficit stress condition was 44, 36 and 46% smaller than the ones in non-water deficit stress condition, respectively. The mean comparison of the families revealed that families number 38, 39, 72, 74 and 119 had high yield in both water deficit stress and non-stress conditions and were recognized as the most drought tolerant families in the population.

In overall, the water deficit stress effect was significant on grain yield. The percent of yield reduction as a result of water deficit stress was highly variable among F₃ families. The biggest reduction (78%) belonged to family number 34 that had the highest grain yield (312 g m⁻²) in non-water deficit stress condition and also the lowest (68 g m⁻²) under water deficit stress condition. This family was identified as the most unstable family among all the F₃ families tested. Family number 7 with the lowest yield reduction (% 4) had average and low yield in water deficit stress and non-stress conditions, respectively. Family number 42 had high and average yield in water deficit stress and non-stress conditions respectively and was recognized as a desirable family for drought environments.

In non-water deficit stress condition the mean of IWUE for F₃ families, KO37 and SP1066 was 0.43, 0.61 and 0.32 g L⁻¹, respectively. These values were 0.33, 0.53 and 0.23 g L⁻¹, respectively, for water deficit stress condition. Among F₃ families, IWUE varied from 0.19 to 0.76 in non-water deficit stress and from 0.11 to 0.56 g L⁻¹ for water deficit stress conditions (Fig. 2). Mean IWUE of F₃ families, KO37 and SP1066 in water deficit stress condition was 23, 11 and 25% smaller than the ones in non-water deficit stress condition, respectively. As it was expected the KO37 parental line was more tolerant to drought stress when compared to the SP1066 parent. Based on this, families number 38, 39, 72, 74 and 119 that had high IWUE in both water deficit stress (average 0.47 g L⁻¹) and non-stress (average 0.63 g L⁻¹) conditions were recognized as the most favorable families in the population. Transgressive segregation in both directions was also observed for grain yield and IWUE in water deficit stress and non-stress conditions (Fig. 2).

Overall the IWUE was reduced by water deficit stress although in some families it was not changed or even increased. For example, families number 42, 50 and 72 had high IWUE in both moisture conditions and their IWUE were reduced by water deficit stress. To date a very few studies have been conducted on assaying IWUE and introducing drought tolerant genotypes in flax (Sharma et al. 2012; Yenpreddiwar et al. 2007).

The mean comparison of parents showed that the most reduction in yield and IWUE traits under drought stress belonged to SP1066 parental genotype. Mean comparison of grain yield and IWUE and their reduction in F₃ families and parental lines under water stress condition, showed that there was a wide diversity among F₃ families. This was an indication that the segregating population had enough polymorphism in terms of drought tolerance.

The ranks of families for SSI and SDI indices were equal. When selecting on these bases, the families with low value of SDI and SSI will be more desirable. A total of 53 families had $SSI > 1$ with families number 27, 32, 34, 36, 45, 46, 55 and 83 showed the highest sensitivity to drought stress. The remaining 68 families had $SSI < 1$ and the lowest SSI in this group belonged to families number 7, 9, 42, 54, 9, 99 and 104. Although these families had the lowest sensitivity to water deficit stress they did not have outstanding performance in non-stress condition, because of their low yield potential.

Base on reverse relationship of SSI to RDI and YSI, the families with $SSI < 1$ and high level of RDI and YSI that had high yield in both water deficit stress and non-stress conditions, including 1, 12, 35, 38, 40, 43, 70, 72, 78, 84, 113 and 117 were introduced as the high yield families with the lowest sensitivity to water deficit stress.

Based on the results, the greater the TOL value, the larger the yield reduction under water deficit stress conditions and the higher sensitivity to water deficit stress. The ranks of families for TOL and SSPI indices were equal. The highest and the lowest value of the indices belonged to families number 7 and 34, respectively. The families with low level of SSI, SDI, TOL and SSPI indices including families number 6, 7, 9, 42, 44, 54, 76, 85, 86, 88, 91, 94, 96, 99, 104, 107 and 114 showed the lowest sensitivity to water deficit stress, but they did not have a high level of yield in non-stress condition. Selection based on these indices detected genotypes with high yield in water deficit stress condition and low yield in non-stress conditions and failed to identifying genotypes with both high yield and drought tolerant characteristics.

The ranks of families for MP, HM, GMP and STI criteria were almost the same. Families number 33, 38, 39, 70, 72, 78 and 119 had high value of these indices and were recognized as high yielding and drought tolerant families. Rating families based on K1STI and K2STI was nearly similar to ones based on STI but families with high K1STI had almost higher yield in non-water deficit stress condition. The opposite was true for the families with high K2STI that had higher yield in stress condition.

Families with high yield in water deficit stress condition showed high YI, the lowest sensitivity to water deficit stress and almost had moderate yield in non-stress condition. The families ranked the same for DI and SNPI criteria. Families number 42, 50, 68, 72, 104 and 118 had high values for these criteria and almost had high and average yield in water deficit stress and non-stress conditions, respectively. Based on ATI, families number 7, 54, 85 and 96 with the lowest level of this index and yield in non-water deficit stress condition, showed the lowest tolerant to drought stress. Families number 26, 27, 31, 32, 33, 34, 35, 36, 37, 38, 39, 45, 47, 59, 81 and 119 with high level of ATI and yield in non-water deficit stress condition showed high tolerance to water deficit stress but almost all of them had average yield in water deficit stress condition. Based on PI index families number 26, 27, 33, 34, 36, 37, 38, 39, 43, 72, 78, 81 and 119 had low level of PI and showed more tolerant to water deficit stress.

Correlation of indices

To determine the most desirable drought tolerance criteria in the population, the correlation coefficient between Y_p , Y_s , $IWUE_p$, $IWUE_s$ and other quantitative indices of drought tolerance were calculated (Table 4). The Y_p was adversely correlated with Y_s ,

depicting that high yield potential under best possible conditions does not anticipate superior yield under water deficit stress conditions. Y_p was highly and positively correlated with IWUE_p, MP, HM, GMP, STI, ATI, SSPI, K1STI and TOL and highly and negatively correlated with PI. Y_s had high and positive correlation with IWUE_s, MP, HM, GMP, STI, YI, DI, SNPI and K2STI and high and negative correlation with TOL, SSI, SSPI, PI and SDI.

Positive correlation of Y_p and negative correlation of Y_s with TOL, SSI, SSPI, PI and SDI criteria suggest that selection based on these indices will result in reduced yield in well - watered conditions. There was higher correlation between MP and Y_p rather than MP and Y_s and between HM and Y_s rather than HM and Y_p . Thus, based on the results, GMP and STI criteria were more useful for prediction of potential yield of the genotypes in both water deficit stress and non-stress conditions, because of very high, positive and significant correlation with IWUE_p, IWUE_s, Y_p and Y_s .

There were positive and significant correlation among IWUE_p, IWUE_s, MP, HM, GMP, STI, ATI, YI, MSTIp and MSTIs. These indices had a weak association to YSI and SDI. The SSI was positively and significantly correlated with SDI, TOL, SSPI and ATI and negatively and significantly correlated with RDI, YSI, DI, SNPI, YI, PI and HM, respectively. So, it seems that SSI, SDI, TOL, SSPI and ATI had the same capability in performing tolerance against stress. The PI was positively correlated with RDI and YSI ($r = 0.29$) and had significant and negative association to other indices with the highest negative correlation belonging to MP ($r = -0.98$) and Y_p ($r = -0.96$). The SNPI had the highest positive correlation with DI.

Biplot analysis

Selection based on a combination of indices may provide a more useful criterion for improving drought resistance of field crops, but study of correlation coefficients are useful in finding the degree of overall linear association between any two attributes. Thus, a better approach such as biplot than a correlation analysis is needed to identify the superior genotypes for both water deficit stress and non-stress conditions. Principal component analysis (PCA) revealed that the first dimension (PC₁) explained 50% of the variation (Table 5). PC₁ was highly and positively correlated with IWUE_p, IWUE_s, Y_p , Y_s MP, HM, GMP, STI, ATI, YI, K1STI and K2STI and negatively correlated with PI. Thus, the first dimension can be named as the yield potential and drought tolerance. Considering the high and positive value of the PC₁ on biplot, genotypes with high values of PC₁ will be high yielding under water deficit stress and non-stress conditions. The second component (PC₂) explained 42.6% of the total variability and was positively correlated with RDI, SNPI, DI and YSI and negatively correlated with SSI, TOL, SDI and SSPI. Therefore, the second component can be named as a stress susceptibility dimension which separates stress-tolerant from non-stress tolerant genotypes. Based on the PC₂, genotypes that their yields were severely affected by water deficit stress are identified.

According to the biplot based on the PC₁ and PC₂, F₃ families with high and positive PC₁ and low and positive PC₂ are suitable for both water deficit stress and non-stress conditions. The F₃ families with high PC₁ and negative PC₂ are suitable for non-stress condition (Fig. 3). The F₃ families with negative PC₁ and PC₂ are unsuitable. In the present study, families number 33, 38, 39, 43,

50, 68, 70, 72, 78, 81, 113, 117, 118 and 119 with high positive PC_1 and low PC_2 were identified as the superior families for both water deficit stress and non-stress conditions. Family number 32 with high positive PC_1 and negative PC_2 , that had high yield potential in non-stress condition and high sensitivity to drought, was identified as the superior family for non-stress condition. In total, 46 families had low or moderate PC_1 and PC_2 , low or moderate yield potential and low sensitivity to water deficit stress. Also 14 families with low PC_1 and high PC_2 , had low yield potential in both water deficit stress and non-stress conditions that defined as the most unstable families.

Classifying families based on STI

The STI values were used to draw three-dimensional graph to find drought resistant genotypes (Fig. 4). According to Fernandez (1992) model, studied families were divided into four categories based on their performance in water deficit stress and non-stress conditions: 22 families positioned in group A that among them families number 26, 33, 36, 37, 38, 39, 43, 70, 72, 78, 81, 113 and 119 had high yield in both water deficit stress and non-stress conditions and high STI criterion (Fig. 5). Families number 27, 31, 32, 34, 45 and 56 were placed in group B having maximum yield in non-water deficit stress condition. Also 56 families situated in group C that among them families number 42, 50, 68 and 118 had the highest yield under water deficit stress condition. The 35 families found to be in group D exhibited low yield in both water deficit stress and non-stress conditions.

Discussion

Different responses of families to moisture conditions indicated that the population have segregated for genes controlling yield and drought tolerance related traits. The parental genotypes SP1066 (Canadian) and KO37 (Iranian) are from two separate gene pools, and were expected to contribute to greater variation. Transgressive segregation observed for grain yield in both moisture conditions, indicated that some of the F_3 families had more drought tolerance than parents (Fig. 2), and have the potential to be used in advanced generations for future breeding programs.

Low correlation between Y_p and Y_s , suggested that high potential yield under optimal conditions does not necessarily result in improved yield under water deficit stress conditions. However, due to genotype by environmental interactions, indirect selection for a drought-prone environment based on the results of optimum conditions will not be efficient. These findings are similar to those previously reported for wheat (Siahpoosh et al. 2011) and barely (Giancarla et al. 2010) that genotypes with low yield potential under well watered conditions had high yield under deficient irrigation conditions.

The estimates of stress tolerance attributes indicated that the identification of drought-tolerant genotypes based on different criteria was varied. Families selected based on SSI, SDI, YSI, RDI, TOL and SSPI showed the lowest sensitivity to water deficit stress, but they did not have the high level of yield in non-stress condition, as previously reported in wheat (Nouraein et al. 2013) and safflower (Majidi et al. 2011). Fernandez (1992) stated that SSI determined sensitive and tolerant families without attention to yield potential. Similar results were reported by Rosielle and Hamblin (1981). Limitations of using SSI, SDI, YSI, RDI, TOL and

SSPI indices have already been described (Lander et al. 1989; Saba et al. 2001; Akhter et al. 2010; Sio-Se Mardeh et al. 2006). Farshadfar et al. (2013a,b) reported that the indices K1STI, K2STI, SSPI, RDI, ATI, SNPI, and DI can be used as the most suitable indicators for screening drought tolerant cultivars. It was suggested that SSI is a useful indicator for wheat breeding where the stress is severe while MP, GMP, TOL HM and STI are better if the stress is less severe (Akçura et al. 2011; Clarke et al. 1984; 1992 and Guttieri et al. 2001).

The families number 33, 38, 39, 70, 72, 78 and 119 with high value of the MP, GMP, HM and STI criteria were introduced as high yielding and drought tolerant families that their advanced generations can be used in future breeding programs of flax. The rank similarity of genotypes and high efficiency of MP, HM, GMP and STI criteria in identifying high yielding and drought tolerant genotypes were previously reported in wheat (Nouraein et al. 2013; Siahpoosh et al. 2011; Ud-Din et al. 1992), barley (Nazari and Pakniat 2010; Giancarla 2010), rice (Akhter et al. 2010), canola (Khalili et al. 2012; Yarnia et al. 2011), corn (Vegh 2013) and safflower (Majidi et al. 2011). Also, relative efficiency of MP, GMP and STI criteria in plant breeding programs for determining drought tolerant families among F_3 and F_4 families in durum wheat (Golabadi et al. 2006) and high yielding F_5 families in rice (Rahimi et al. 2013) was previously reported.

Based on the correlation coefficient among Y_p , Y_s , $IWUE_p$, $IWUE_s$ and other quantitative indices of drought tolerance (Table 4), GMP and STI criteria were more useful for prediction of potential yield in both water deficit stress and non-stress conditions, because of very high, significant and positive correlation with $IWUE_p$, $IWUE_s$, Y_p and Y_s . The results of present study were congruent with those reported in common bean (Ramirez and Kelly 1998), wheat (Nouraein et al. 2013), soybean (Yan and Rajcan 2002), safflower (Pourdad 2008; Majidi et al. 2011) and canola (Yarnia et al. 2011). Low correlation among some indices stated that each index may be a potential indicator of different biological response to water deficit stress (Farshadfar et al. 2013a).

Selection based on a combination of indices may provide a more useful criterion for improving drought resistance in plants. Biplot of genotypes based on two PCs obtained from PCA of their drought tolerance indices is an acceptable method to identify superior genotypes under water deficit stress and non-stress environments. The correlation coefficient between two indices is almost angle cosine of their vectors. In biplot of genotypes based on drought tolerance indices, the cosine of the angle between the vectors of two indices approximates the correlation coefficient between them.

The PCA was performed to group the drought tolerance indices based on their function as well as the genotypes using their drought tolerance indices. In the present study, because of high and positive correlation and the least angle among GMP and STI indices with Y_p and Y_s , they were introduced as the most suitable selection criteria. Since, in this study, the first two PC explained 92.6% of the total variability, the biplot based on the PC_1 and PC_2 that scattered all F_3 families and indices was a useful tool to evaluate indices and to identify high yielding - drought tolerant families. Based on this, families number 33, 38, 39, 43, 50, 68, 70, 72, 78, 81, 113, 117 118 and 119 were identified as the superior families for water deficit stress and non-stress conditions. In order to

select based on a combination of indices, some researchers have used PCA (Majidi et al. 2011; Ebrahimiyan et al. 2012) and observed that genotypes with larger PC_1 and lower PC_2 scores had high yields (stable genotypes), and genotypes with lower PC_1 and larger PC_2 scores had low yields (unstable genotypes). Similar results were observed in assaying drought tolerance in segregating populations of durum wheat (Golabadi et al. 2006) and rice (Rahimi et al. 2013).

According to Fernandez (1992) model, the best possible selection criteria should distinguish genotypes of Group A (genotypes expressing uniform superiority in both water deficit stress and non-stress environments) from the genotypes of other three groups. Based on this, some researchers introduced GMP and STI criteria as the best indices for identifying tolerant genotypes (Farshadfar et al. 2013; Majidi et al. 2011; Akhter et al. 2010). Nouraein et al. 2013) reported that GMP is more powerful in separating group A and has a lower susceptibility to different amounts of Y_s and Y_p , so; GMP will be bias when the difference between Y_s and Y_p is less.

In this study, the results of triplot analysis based on STI index and grain yield in both water deficit stress and non-stress conditions introduced families number 26, 33, 36, 37, 38, 39, 43, 70, 72, 78, 81, 113 and 119 as the most favorable families for drought tolerance (Fig. 4 and 5). Families number 27, 31, 32, 34, 45 and 56 and families number 42, 50, 68 and 118 were recommended for non-water deficit stress and stress conditions, respectively.

Conclusion

In this study, the presence of high variability and transgressive segregation for grain yield and IWUE in parental lines (KO37 and SP1066) and F_3 families under both water deficit stress and non-stress conditions indicated that the F_3 population or its advanced generations can be used in selection programs, increasing drought tolerance and identification of QTL related to agronomically important traits in flax. Based on the result from assessing the selection criteria, GMP and STI indices that had positive significant correlations with both Y_p and Y_s is suggested as desirable indices to identify drought tolerant genotypes. The results of biplot and triplot analysis, based on STI index, Y_p and Y_s , indicated that families number 26, 33, 36, 37, 38, 39, 43, 70, 72, 78, 81, 113 and 119 had the best combination of yield production under water deficit stress and non-water deficit stress conditions and therefore further validation is required to introduce them into flax breeding programs in Iran and elsewhere.

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Table1- The weather conditions of the Research Farm of Isfahan University of Technology (RFIUT) during the vegetation period in 2012.

Months	Cumulative Sunshine (hour)	Cumulative pan evaporation (mm)	Cumulative rain (mm)	Average of air humidity (%)	Average of air temperature (°C)
March	210.5	125	7.8	48.6	10.6
April	252.0	159.1	38.4	44.4	13.1
May	288.1	250.4	10	36.0	19.4
Jun	288.1	250.4	0	36.0	19.4
July	357.3	376	0	22.7	28.1
Aug	334.9	275.8	0	25.1	24.9

Table2- Relationships of 16 drought tolerance indices

No.	Index	Ab.	Formula ^b	Explanation	Reference
1	Stress susceptibility index	SSI	$(1 - (Y_s/Y_p)) / (1 - (\bar{Y}_s/\bar{Y}_p))$	The genotypes with $SSI < 1$ have high level of yield in stress conditions and are more resistant to drought stress conditions, but almost the genotypes do not have the high level of yield in non-stress condition.	Fischer and Maurer 1978
2	Relative drought index	RDI	$(Y_s/Y_p) / (\bar{Y}_s/\bar{Y}_p)$		Fischer and Wood 1979
3	Tolerance index	TOL	$Y_p - Y_s$		
4	Mean productivity	MP	$(Y_p + Y_s)/2$	The genotypes with low values of TOL and high value of MP and HM are more stable in different conditions.	Rosielle and Hamblin 1981
5	Harmonic mean	HM	$[2(Y_s * Y_p)/(Y_s + Y_p)]$		
6	Stress tolerance index	STI	$(Y_p * Y_s)/\bar{Y}_p^2$	The genotypes with high STI values will be tolerant to drought stress	Fernandez 1992
7	Geometric mean productivity	GMP	$\sqrt{(Y_p * Y_s)}$	GMP is interested in relative performance under various conditions, since drought stress can vary in severity in the field environment over years.	Fernandez 1992
8	Yield index	YI	$Y_s/\bar{Y}_s$		Gavuzzi et al. 1997
9	Yield stability index	YSI	Y_s/Y_p		Bousslama and Schapaugh 1984
10	Drought resistance index	DI	$Y_s * (Y_s/Y_p)/\bar{Y}_s$		Lan 1998
11	Modified stress tolerance index	MSTI ^a	$K_iSTI, k_1 = \frac{Y_p^2}{\bar{Y}_p^2}, k_2 = \frac{Y_s^2}{\bar{Y}_s^2}$		Farshadfar and Sutka 2002
12	Abiotic tolerance index	ATI	$[(Y_p - Y_s)/(\bar{Y}_p/\bar{Y}_s)] * \sqrt{(Y_p * Y_s)}$	They were introduced to identify relatively tolerant (through ATI and SSPI) and resistant (through SNPI) genotypes under non irrigated and irrigated conditions	Moosavi et al. 2008
13	Stress susceptibility percentage index	SSPI	$[(Y_p - Y_s)/(2 * \bar{Y}_p)] * 100$		
14	Stress non-stress production index	SNPI	$\sqrt[3]{[(Y_p + Y_s)/(Y_p - Y_s)] * (Y_p * Y_s * Y_s)}$		
15	Sensitivity drought index	SDI	$Y_p - Y_s/Y_p$		Farshadfar et al. 2012
16	Superiority index	P _i	$\sum_{j=1}^n (X_{ij} - M_j)^2 / 2n$		Clarke et al. 1992; Lin et al. 1986

^a where k_i is the correction coefficient

^b In the above formulas, $Y_s, Y_p, \bar{Y}_s$ and $\bar{Y}_p$, represent yield under stress, yield under non-stress for each genotype, and yield mean in stress and non-stress conditions for all genotypes, respectively. n, X_{ij} and M_j are the number of environments which is equal two, the grain yield of i th genotype in the j th environment and the yield of the genotype with maximum yield at environment j , respectively.

Table3- Analysis of variance based on RCBD for grain yield (Y) and irrigation water use efficiency (IWUE) in non- stress (p) and stress (s) conditions and studied indices

S.O.V.	df	Mean Square ^a of Index ^b						
		IWUEp	IWUEs	Yp	Ys	SSI	RDI	TOL
Block	2	0.62**	0.62**	103743**	55111**	1.62**	0.98**	10213**
Family	120	0.03**	0.02**	5697**	1834**	0.34**	0.21**	4831**
Error	240	0.02	0.01	2602	854	0.17	0.11	2914
S.O.V.	df	MP	HM	STI	GMP	YI	YSI	DI
Block	2	76874**	66776**	0.31**	70364**	5.70**	0.31**	3.91**
Family	120	2558**	2208**	0.19**	2259**	0.19**	0.07**	0.20**
Error	240	999	887	0.09	867	0.09	0.03	0.11
S.O.V.	df	K1STI	K2STI	ATI	SSPI	SNPI	PI	SDI
Block	2	83.47**	74.52**	521868818**	833**	360372**	4995980729**	0.31**
Family	120	4.20 ^{ns}	2.99 ^{ns}	52646394**	394**	16277**	231747267**	0.07**
Error	240	3.67	2.37	36389911	238	11053	78518391	0.03

^a **, * and ns: Significant at 5% and 1% probability levels and non-significant, respectively

^b SSI: Stress susceptibility index, RDI: Relative drought index, TOL: Tolerance index, MP: Mean productivity, HM: Harmonic mean, STI: Stress tolerance index, GMP: Geometric mean productivity, YI: Yield index, YSI: Yield stability index, DI: Drought resistance index, MSTI: Modified stress tolerance index, ATI: Abiotic tolerance index, SSPI: Stress susceptibility percentage index, SNPI: Stress non-stress production index, SDI: Sensitivity drought index and P_i: Superiority

Table4- Correlation coefficients of grain yield (Y) and irrigation water use efficiency (IWUE) in non- stress (IWUEp) and stress (IWUEs) conditions and studied indices

Index ^b	IWUEp	IWUEs	Yp	Ys	SSI	RDI	TOL	MP	HM	STI
IWUEs	0.40**									
Yp	0.97**	0.41**								
Ys	0.38**	0.95**	0.42**							
SSI	0.48**	-0.48**	0.47**	-0.53**						
RDI	-0.48**	0.48**	-0.47**	0.53**	-1.00					
TOL	0.82**	-0.13	0.83**	-0.16	0.84**	-0.84**				
MP	0.89**	0.71**	0.92**	0.74**	0.13	-0.13	0.55**			
HM	0.63**	0.91**	0.67**	0.94**	-0.24**	0.24**	0.15	0.90**		
STI	0.78**	0.80**	0.82**	0.82**	-0.02	0.02	0.38**	0.96**	0.95**	
GMP	0.76**	0.84**	0.80**	0.87**	-0.07	0.07	0.34**	0.97**	0.98**	0.98**
YI	0.38**	0.95**	0.42**	1.00**	-0.53**	0.53**	-0.16**	0.74**	0.94**	0.82**
YSI	-0.48**	0.48**	-0.47**	0.53**	-1.00**	1.00**	-0.84**	-0.12	0.24	0.02
DI	-0.03	0.81**	-0.02	0.87**	-0.84**	0.84**	-0.56**	0.36**	0.66**	0.48**
K1STI	0.77**	0.36**	0.79**	0.37**	0.25**	-0.25**	0.63**	0.74**	0.55**	0.76**
K2STI	0.51**	0.72**	0.53**	0.74**	-0.14	0.14	0.12	0.71**	0.79**	0.86**
ATI	0.89**	0.13	0.92**	0.12	0.60**	-0.60**	0.92**	0.73**	0.41**	0.62**
SSPI	0.82**	-0.13	0.83**	-0.16	0.84**	-0.84**	1.00**	0.55**	0.15	0.38**
SNPI	0.03	0.75**	0.05	0.82**	-0.77**	0.77**	-0.45**	0.38**	0.62**	0.47**
PI	-0.92**	-0.61**	-0.96**	-0.62**	-0.29**	0.29**	-0.65**	-0.98**	-0.83**	-0.90**
SDI	0.48**	-0.48**	0.47**	-0.53**	1.00**	-1.00**	0.84**	0.12	-0.24**	-0.02
	GMP	YI	YSI	DI	K1STI	K2STI	ATI	SSPI	SNPI	PI
YI	0.87**									
YSI	0.07	0.53**								
DI	0.54**	0.87**	0.84**							
K1STI	0.65**	0.37**	-0.25**	0.04						
K2STI	0.77**	0.74**	0.14	0.53**	0.67**					
ATI	0.57**	0.12	-0.60**	-0.30**	0.85**	0.35**				
SSPI	0.34**	-0.16	-0.84**	-0.56**	0.63**	0.12	0.92**			
SNPI	0.52**	0.82**	0.77**	0.94**	0.09	0.50**	-0.21**	-0.45**		
PI	-0.92**	-0.62**	0.29**	-0.21*	-0.67**	-0.61**	-0.77**	-0.65**	-0.24**	
SDI	-0.07	-0.53**	-1.00**	-0.84**	0.25**	-0.14	0.60**	0.84**	0.77**	-0.29**

^a ** and * Significant at 5% and 1% probability levels

^b SSI: Stress susceptibility index, RDI: Relative drought index, TOL: Tolerance index, MP: Mean productivity, HM: Harmonic mean, STI: Stress tolerance index, GMP: Geometric mean productivity, YI: Yield index, YSI: Yield stability index, DI: Drought resistance index, MSTI: Modified stress tolerance index, ATI: Abiotic tolerance index, SSPI: Stress susceptibility percentage index, SNPI: Stress non-stress production index, SDI: Sensitivity drought index and P_i: Superiority index

Table5- The coefficients of grain yield (Y) and irrigation water use efficiency (IWUE) in non- stress (**IWUE_p**) and stress (**IWUE_s**) conditions and studied indices in PC₁ and PC₂ based on principal component analysis.

PC	Eigen Value	% Cumulative	Eigen Vectors of index ^a										
			IWUE _p	IWUE _s	Y _p	Y _s	SSI	RDI	TOL	MP	HM	STI	GMP
PC ₁	10.5	50.1	0.26	0.24	0.27	0.25	0.01	-0.01	0.14	0.30	0.29	0.30	0.30
PC ₂	8.9	92.7	-0.16	0.18	-0.16	0.20	-0.33	0.33	-0.29	-0.03	0.10	0.02	0.04
PC	Eigen Value	% Cumulative	YI	YSI	DI	K1STI	K2STI	ATI	SSPI	SNPI	PI	SDI	
PC ₁			0.25	-0.01	0.14	0.23	0.25	0.21	0.14	0.14	-0.29	0.01	
PC ₂			0.20	0.33	0.30	-0.10	0.07	-0.22	-0.29	0.27	0.08	-0.33	

^a SSI: Stress susceptibility index, RDI: Relative drought index, TOL: Tolerance index, MP: Mean productivity, HM: Harmonic mean, STI: Stress tolerance index, GMP: Geometric mean productivity, YI: Yield index, YSI: Yield stability index, DI: Drought resistance index, MSTI: Modified stress tolerance index, ATI: Abiotic tolerance index, SSPI: Stress susceptibility percentage index, SNPI: Stress non-stress production index, SDI: Sensitivity drought index and P_i: Superiority index

Fig. 1- The amount of cumulative total applied water (irrigation and rain) (mm) under water deficit stress and non-water stress conditions, cumulative reference evapotranspiration (ET₀) and cumulative flax evapotranspiration (ET_c) in growing season 2012

Fig. 2- Frequency distribution of F₃ families derived from a cross between KO37 and SP1066 for A) grain yield (g m⁻²) and B) irrigation water use efficiency (IWUE, g Lit⁻¹) in stress and non stress conditions. Average yield of KO37 and SP1066 are indicated on the graph in both stress and non-stress conditions.

Fig. 3- Biplot analysis of F3 families based on PC₁ and PC₂. The vector was drawn from center to each index.

Yp: grain yield in non- stress and Ys: in stress conditions, IWUEp: irrigation water use efficiency in non- stress and **IWUEs** : in stress conditions, SSI: Stress susceptibility index, RDI: Relative drought index, TOL: Tolerance index, MP: Mean productivity, HM: Harmonic mean, STI: Stress tolerance index, GMP: Geometric mean productivity, YI: Yield index, YSI: Yield stability index, DI: Drought resistance index, MSTI: Modified stress tolerance index, ATI: Abiotic tolerance index, SSPI: Stress susceptibility percentage index, SNPI: Stress non-stress production index, SDI: Sensitivity drought index and P_i: Superiority index

Fig. 4- Triplot analysis of F3 families based on grain yield in non- stress (Yp) and stress (Ys) conditions and Stress tolerance index (STI). The vector was drawn from each family to bottom. Families were classified to four groups.

Fig.5- Mean of grain yield (GY) and irrigation water use efficiency (IWUE) among superior F₃ families in stress (S) and non stress (N) conditions. Numbers 1 to 15 on horizontal axis corresponds to families number 26, 36, 37, 38, 39, 43, 70, 72, 74, 78, 81, 113.119, KO37 and SP1066 respectively.